

PHYLOGENETIC RELATIONSHIPS IN THE *CORYLOPSIS*
COMPLEX (HAMAMELIDACEAE): EVIDENCE FROM
SEQUENCES OF THE INTERNAL TRANSCRIBED
SPACERS OF NUCLEAR RIBOSOMAL DNA AND
MORPHOLOGY

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ABSTRACT. Morphological and nuclear ribosomal Internal Transcribed Spacer (ITS) sequence data were used to examine relationships among the genera in the *Corylopsis* complex, comprising *Corylopsis*, *Distylium*, *Eustigma*, *Fortunearia*, and *Sinowilsonia*. The ITS-2 based cladogram had the lowest resolution, whereas the ITS-1 and the pooled data of ITS-1 and ITS-2 produced similar phylogenies with complete resolution of intergeneric relationships within the complex. Both morphological data and ITS DNA sequences support separation of *Corylopsis* from the other genera, and a closer relationship of *Sinowilsonia* to *Fortunearia* than to *Distylium*. The monophyly of *Eustigma-Fortunearia-Sinowilsonia* was supported by the ITS sequence data. This study substantiates Endress's recent interpretation of the intergeneric relationships in the *Corylopsis* complex. The ITS phylogeny also supports the hypothesis that floral structures in members of the *Corylopsis* complex appear to have been modified for wind pollination in the course of evolution.

Key Words: phylogeny, *Corylopsis* complex, Hamamelidaceae, ITS DNA sequences

The Hamamelidaceae, consisting of 31 genera and about 140 species, is widely distributed in subtropical and temperate areas of both the Old and New Worlds (Endress 1993; Zhang and Lu 1995). Morphological diversity in this family, especially of reproductive attributes, has prompted numerous systematic studies (Bogle 1970, 1986, 1987, 1989, 1990; Bogle and Philbrick 1980; Chang 1973, 1979; Endress 1967, 1970, 1976, 1977, 1989a, b, c; Harms 1930; Mione and Bogle 1990; K. Tong 1930; Y. Tong 1943). Although subfamilial classification of the family has been, and is still, con-

troversial, the subfamily Hamamelidoideae has been widely accepted as a monophyletic clade (Bogle and Philbrick 1980; Endress 1989a, b). The most definitive characteristics of this subfamily are uniovulate carpels, 2-carpellate capsules, and a ballistic mechanism for seed dispersal (Endress 1989a). However, the phylogenetic relationships among some members of the Hamamelidoideae remain problematic. One unresolved problem is that of the relationships of the genera that have been considered more or less related to *Corylopsis* Sieb. et Zucc. The *Corylopsis* complex for the purpose of this study encompasses five well-defined genera: *Corylopsis*, *Distylium* Sieb. et Zucc., *Eustigma* Gardner et Champ., *Fortunearia* Rehd. et Wils., and *Sinowilsonia* Hemsl.

The *Corylopsis* complex shows a high diversity of floral structures and can be considered as an epitome of the family. For example, flowers have conspicuous sepals and petals in *Corylopsis*; petals are rather reduced in *Eustigma*, *Fortunearia*, and *Sinowilsonia*; there are no sepals or petals in *Distylium*. Therefore, resolution of the intergeneric relationships within the complex may shed light on the evolutionary directionalities of floral characters in the Hamamelidaceae.

Harms (1930) made the first comprehensive taxonomic treatment of the Hamamelidaceae, placing 21 genera in five subfamilies (two additional genera that were little known were not placed). The Hamamelidoideae was the largest subfamily, containing 16 genera in five tribes. The genera of the *Corylopsis* complex fell into three of these tribes: the monogeneric Eustigmatae (*Eustigma*); the Corylopsideae (*Corylopsis* and *Fortunearia*), and the Distylieae (*Distylium*, *Sinowilsonia*, and *Sycopsis*).

Schulze-Menz (1964) basically adopted Harms's classification, but moved *Sinowilsonia* out of the tribe Distylieae and placed it close to *Fortunearia* of the Corylopsideae. Many botanists have followed Harms's system at the tribal level (e.g., K. Tong 1930; Chang 1973, 1979).

Endress (1989a, b), however, moved both *Fortunearia* and *Sinowilsonia* into the Eustigmatae, thus treating the Corylopsideae as a monogeneric tribe. Nonetheless, as admitted by Endress (1989a), the phylogenetic relationships among the genera were not well resolved.

Sequences of the Internal Transcribed Spacer (ITS) of nuclear ribosomal DNA have been widely and almost routinely used for phylogenetic studies of angiosperms at specific and generic levels

Table 1. Vouchers for the species sampled. AA, the Arnold Arboretum; GUNH, the Greenhouse at the University of New Hampshire; WI, Woodlanders Inc., SC. Each species name is followed by a 3-letter acronym used in other tables and figures.

Species	Voucher	Source	GenBank Accessions
<i>Corylopsis pauciflora</i> (cpa)	Li01	AA	U65462
<i>C. sinensis</i> (csi)	Li02	AA	U65461
<i>C. spicata</i> (csp)	Li03	AA	U65463
<i>Distylium myricoides</i> (dim)	Bogle	GUNH, WI	U65464
<i>D. racemosum</i> (dir)	Bogle	GUNH, WI	U65465
<i>Eustigma oblongifolia</i> (eus)	Chung, N. J.	Taiwan	U65466
<i>Fortunearia sinensis</i> (for)	Li04	AA	U65467
<i>Sinowilsonia henryi</i> (sin)	Li05	AA	U65468
<i>Mytilaria laosensis</i> (myt)	Luo, Y. C.	Xinning, China	U65469

(Baldwin et al. 1995; Campbell et al. 1995; Suh et al. 1993; Wojciechowski et al. 1993). In this study, therefore, we based our analysis of relationships on the evidence from both the ITS and morphological data. The objective of this investigation has been to evaluate the alternative hypotheses concerning the relationships among the genera of the complex, which can be translated into the following questions: 1) Should *Corylopsis* be isolated from the other genera and be treated as a monogeneric tribe? 2) Is it reasonable to put *Eustigma* together with *Fortunearia* and *Sinowilsonia* in the Eustigmateae even though *Eustigma* is somewhat different from the others morphologically and geographically? and 3) Is *Sinowilsonia* more closely related to *Distylium* than to *Fortunearia*?

MATERIALS AND METHODS

The internal transcribed spacers ITS-1 and ITS-2 were sequenced for nine species, representing all five genera of the *Corylopsis* complex and an outgroup, *Mytilaria* Lecomte (Table 1). Three of the six genera sampled are monotypic, including *Fortunearia*, *Mytilaria*, and *Sinowilsonia*. Several to many species have been described in *Corylopsis* (9–20), *Distylium* (10–18), and *Eustigma* (2–4; Zhang and Lu 1995). These three genera, however, have not been monographed in recent years and the number of valid species is debatable. Furthermore, materials of some species were unavailable for this study. Therefore, the representation of these three genera is unavoidably limited to generally accepted,

valid species for which samples could be obtained from cultivated plants or from accessible field locations.

The genera of the *Corylopsis* complex represent three of the four tribes in the monophyletic Hamamelidoideae, but the tribal relationships are unresolved (Endress 1989a). Thus, it is uncertain which genera should be used as outgroups from within the Hamamelidoideae. Endress (1989a) used Exbucklandioideae (incl. *Disanthus*, *Mytilaria*, and *Exbucklandia*) as the outgroup in his phylogenetic evaluation of the Hamamelidoideae based on morphological data. Our preliminary results from sequences of *matK* gene encoding plastid maturase suggested that the most closely related genera to the Hamamelidoideae were *Mytilaria* and *Disanthus* (unpubl.). Therefore, we chose *Disanthus* and *Mytilaria* as outgroups. Although we obtained the same tree topology when using either or both of these two genera as outgroups, the confidence of the sequence alignment decreased when both *Mytilaria* and *Disanthus* were used. Thus, we included only *Mytilaria* in the parsimony analysis. The DNA sequences obtained in this study have been submitted to the GenBank and their accession numbers are listed in Table 1 (*Disanthus* is not included).

Molecular techniques. Total genomic DNAs were extracted from young leaves using the standard DNA extraction procedures of Doyle and Doyle (1987). The universal primers ITS4 and ITS5 of White et al. (1990) were used to amplify the entire ITS nuclear DNA region using the Polymerase Chain Reaction[™] (PCR). Each 50 μ l reaction included 4–5 units of *Taq* (Promega, Madison, WI), 4–5 units of *Taq* Extender (Stratagene, La Jolla, CA), 1 \times *Taq* extender buffer, 2.5 mM dNTPs, 50–100 ng DNA, and 20 μ M primers. Amplifications were preceded by a three minute 94°C Hot Start (D'Aquila et al. 1991) and conducted in thin-walled tubes in a MJ-Research thermocycler (Watertown, MA). The PCR program consisted of 30 cycles with 94°C denaturation for 30 seconds, 45°C annealing for 115 seconds, and 72°C extension for 115 seconds. The final cycle was followed by a ten minute extension step at 72°C.

The PCR amplified products were purified on 1.1% low melting point agarose gels in 1 \times TBE buffer (pH 8.0). The ITS bands of about 740 bp, identified by comparison to Φ X174 *Hae*III DNA size markers, were excised and agarase-digested for 30 minutes. Then the purified PCR products were used as templates for direct

double-stranded sequencing using cycle sequencing and dye-di-deoxynucleotide terminator chemistry reactions, and the products were resolved on an ABI 373A fluorescent sequencer (Applied Biosystems, Foster City, CA). The procedures were carried out according to the manufacturer's instructions at the University of New Hampshire Sequencing Facility Center. Besides ITS4 and ITS5, ITS2 and ITS3 of White et al. (1990) were used as internal sequencing primers.

DNA sequence chromatograms were analyzed using the *Seqed* program (Applied Biosystems) and the sequences were then contigged using the *Seqman* of DNA* software package (Madison, WI). The boundaries of ITS-1 and ITS-2 were determined by comparing the limits of the 3' end of the 18S, 5.8S, and 5' end of the 26S rRNA sequences of the ITS region in *Canella winterana* (GenBank accession number: L03844).

Statistical analysis. Chi-square independence tests were applied to test whether the distribution of base compositions was significantly different among the genera. Analysis of variance (ANOVA) was utilized to test the null hypothesis that pairwise divergences among the genera were significantly different between ITS-1 and ITS-2 (Zar 1996).

Morphological data. Twenty-three morphological characters for the *Corylopsis* complex and the outgroup *Mytilaria* were collected based on literature and our own observations (Bogle 1990; Bogle and Philbrick 1980; Chang 1979; Endress 1989b; Li and Bogle 1995; Li et al. 1993; Y. Tong 1943). The characters and their states are described in Table 2. We included one species for each genus in the morphological analysis, since little intrageneric variation in the collected morphological characters resulted in identical scores for species within a genus. As a result, the number of species included in the morphological data set is smaller than the number of species in the ITS data set.

Phylogenetic analyses. The DNA sequences were aligned using the clustal option of the *Megalign* program of DNA*. The aligned sequences are available from the authors. All DNA sequence characters and their states were unweighted, and indels were coded as missing data. Phylogenies were reconstructed using PAUP 3.1.1. (Swofford 1993) with the exhaustive search option.

Table 2. Morphological characters and their states used in the parsimony analysis.

Character	State and Code
1. Seed dispersal	Nonballistic (0), ballistic (1)
2. Venation	Pinnate (0), intermediate (1), palmate (2)
3. Number of ovules per carpel	>3 ovules (0), 3 ovules (1), 1 ovule (2)
4. Nodal anatomy	Trilacunar (0), multilacunar (1)
5. Sexuality	Bisexual (0), andromonoecious (1), monoecious (2)
6. Petals	Distinct (0), reduced (1), absent (2)
7. Pollen apertures	Tricolpate (0), tetracolpate (1)
8. Pollination	Insect (0), wind (1)
9. Pollen surface	Smooth (0), verrucate (1)
10. Ovary position	Semi-inferior (0), superior (1)
11. Stipules	Filamentous (0), leafy (1)
12. Trichome types	Scale (0), stellate (1)
13. Stigma	Unexpanded (0), strongly expanded (1)
14. Habit	Evergreen (0), deciduous (1)
15. Inflorescence	Spike (0), spadix (1)
16. Staminodes	Present (0), absent (1)
17. Anther connective protrusion	Absent (0), present (1)
18. Filament	Longer than or equal to anther (0), shorter than anther (1)
19. Vessel bars	More than 30 (0), 30 or fewer (1)
20. Crystal types	Absent (0), simple (1), cluster (2)
21. Flower parts	Fixed (0), variable (1)
22. Foliar sclereids	Absent (0), fusiform (1), libriform (2)
23. Lenticel on ovary	Absent (0), present (1)

One thousand replicate bootstrap and decay analyses were performed to obtain the indices of relative support for individual clades. The morphological characters were treated as unordered and their states were unweighted to avoid biases in the parsimony analysis. The tree generated based on the combined ITS and morphology data sets was imported into the MacClade 3.03 program (Maddison and Maddison 1992) to analyze the unambiguous changes of the morphological characters along the branches.

RESULTS

Sequence characteristics. Sequence lengths in the *Corylopsis* complex ranged from 236 to 275 bases in ITS-1 and from 224 to

238 bases in ITS-2. ITS-1 was longer than ITS-2 for all taxa sampled except *Eustigma*, *Fortunearia*, and *Sinowilsonia*, whose two spacers were more or less equal in length. GC contents for ITS-1 and ITS-2 were very similar, 57–64% and 61–67%, respectively (Table 3). An Independence test showed that distribution of base compositions (A, T, G, C, and GC content) was not significantly different for all the genera ($P > 0.8$).

The pairwise divergences among the genera ranged from 3.4% to 17.8% in ITS-1 and from 6% to 14.9% in ITS-2, and divergences between these genera and the outgroup *Mytilaria* varied slightly from 21.6% to 25.8% in the ITS sequences. In *Corylopsis* and *Distylium*, where more than one species was sampled, the divergences within each genus were generally lower than 4% (Table 3). Analysis of variance (ANOVA) demonstrated that pairwise divergences between ITS-1 and ITS-2 were not significantly different ($P = 0.8$), nor were the pairwise divergences among the genera in the combined data of ITS-1 and ITS-2 ($P = 0.1$).

Alignment of the sequences required 14 indels, six of which were two or more bases in length. Table 4 lists the largest six indels in these ITS sequences. Noticeably, deletion 4 occurred only in *Corylopsis*, whereas deletion 2, consisting of 36 bases, was found in *Eustigma*, *Fortunearia*, and *Sinowilsonia*. These three genera also had deletion 3 in common with *Corylopsis*. Both species of *Distylium* sampled possessed deletion 5, and shared deletion 6 with *Corylopsis pauciflora*, *Eustigma*, *Fortunearia*, and *Sinowilsonia*. None of the six indels was found in *Mytilaria*.

Phylogenetic trees. When only ITS-1 sequences were utilized in the parsimony analysis, an exhaustive search found one shortest tree of 131 steps (Figure 1a) in which a clade of *Corylopsis* and a clade of *Distylium-Eustigma-Fortunearia-Sinowilsonia* were well resolved, with bootstrap values and decay indices of 100%, 11 steps, and 98%, six steps, respectively. However, *Eustigma* and *Fortunearia* were grouped within a clade weakly supported by a 60% bootstrap value and one step of decay.

The strict consensus tree of the two shortest trees of 114 steps based on ITS-2 data (Figure 1b) did not resolve the relationships of the three groups, *Corylopsis*, *Distylium*, and *Eustigma-Fortunearia-Sinowilsonia*. However, the internal relationships of the three clades were basically the same as in the ITS-1 phylogeny,

Table 3. Sequence length, GC content and divergences of ITS-1 and ITS-2 in the sampled species (above diagonal ITS-2, below diagonal ITS-1; Taxon abbreviations as in Table 1).

Species	GC										GC Content		
	Length	Content	<i>cpa</i>	<i>csi</i>	<i>csp</i>	<i>dim</i>	<i>dir</i>	<i>eus</i>	<i>for</i>	<i>sin</i>		<i>myt</i>	Length
<i>cpa</i>	269	59.85	0	3	3.9	11.7	12.6	12	11.2	14.9	22.7	233	63.09
<i>csi</i>	274	57.66	4.1	0	0.9	10.4	10.8	11.2	10.7	12.9	22.6	234	64.95
<i>csp</i>	275	57.45	3.7	2.2	0	11.3	11.7	12	11.6	13.7	22.1	234	64.11
<i>dim</i>	271	62.21	15.8	17.8	17.7	0	1.7	9.9	8.2	12.8	22	235	65.95
<i>dir</i>	271	64.21	15.8	17.8	17.7	1.1	0	9.4	8.5	12.8	21.6	235	66.81
<i>eus</i>	236	59.74	13.5	15.4	15.3	8.5	8.9	0	6	9.4	22.5	235	64.25
<i>for</i>	236	62.71	13.9	16.6	16.5	9.3	9.7	5.1	0	7.6	21.9	236	65.26
<i>sin</i>	236	60.16	11.7	14.5	14.4	8.5	8.9	3.4	3.8	0	25.8	238	65.18
<i>myt</i>	275	63.64	22.7	25	24.5	23.4	23	24	25.2	24.8	0	224	61.34

Table 4. Indels of two or more bases in length of ITS DNA sequences in the sampled species (+ presence; - absence; * deletion number; ** base range; Taxon abbreviations as in Table 1).

Species	1* 63-67**	2 110-145	3 302-323	4 334-337	5 338-339	6 490-491
<i>cpa</i>	+	-	+	+	-	+
<i>csi</i>	-	-	+	+	-	-
<i>csp</i>	-	-	+	+	-	-
<i>dim</i>	-	-	-	-	+	+
<i>dir</i>	-	-	-	-	+	+
<i>eus</i>	-	+	+	-	-	+
<i>for</i>	-	+	+	-	-	+
<i>myt</i>	-	-	-	-	-	-
<i>sin</i>	-	+	+	-	-	+

with the exception of *Fortunearia* and *Sinowilsonia* forming a clade supported by a bootstrap value of 61% and a decay index of one step.

When ITS-1 and ITS-2 were combined in the analysis, a single shortest tree of 246 steps was generated (Figure 1c), showing relationships similar to those of the ITS-1 tree, but with *Fortunearia* and *Sinowilsonia* forming a clade sister to *Eustigma*. The combination of the two spacers resulted in stronger support for the group of *Eustigma*, *Fortunearia*, and *Sinowilsonia* relative to either of the separate data sets.

The single shortest phylogenetic tree (34 steps) produced, based on morphological data (Figure 2), contained two clades in the complex: *Corylopsis* and *Eustigma-Distylium-Fortunearia-Sinowilsonia*. Within the second clade, *Eustigma* was the basal genus followed by *Distylium*, and sister to *Distylium* was the clade of *Fortunearia* and *Sinowilsonia*. The bootstrap value and decay index were 75% and two steps for the clade of *Eustigma-Distylium-Fortunearia-Sinowilsonia*, and these supporting values were all small for the internal relationships.

A parsimony analysis using the combined data set of morphology and ITS sequences produced the same cladogram (Figure 3) as the combined ITS phylogeny (Figure 1c).

As shown in Figure 3, mapping unambiguous changes of the morphological characters on the tree that was generated using the combined ITS and morphological data revealed that the clade of *Distylium* and *Eustigma-Fortunearia-Sinowilsonia* was

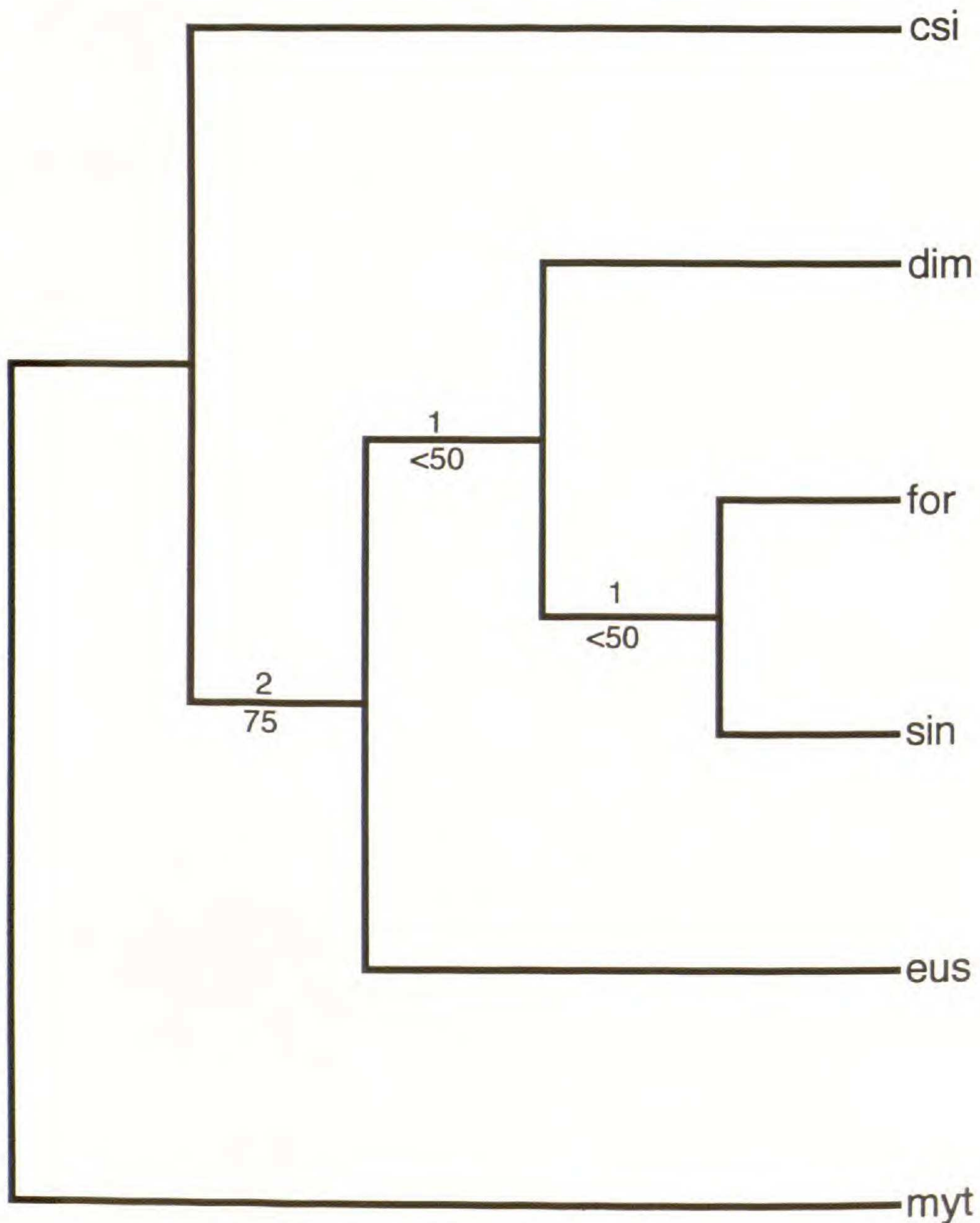


Figure 2. The single shortest tree of 34 steps based on morphology. CI = 0.824, RI = 0.538. Numbers above branches indicate decay index values, and numbers below branches are bootstrap percentages. Taxon abbreviations as in Table 1.

supported by three unambiguous character state changes: pollination from entomophilous to anemophilous, stipules from leafy to filamentous, and staminodes from present to absent. *Fortunearia* and *Sinowilsonia* share deciduousness and absence of foliar crystals.

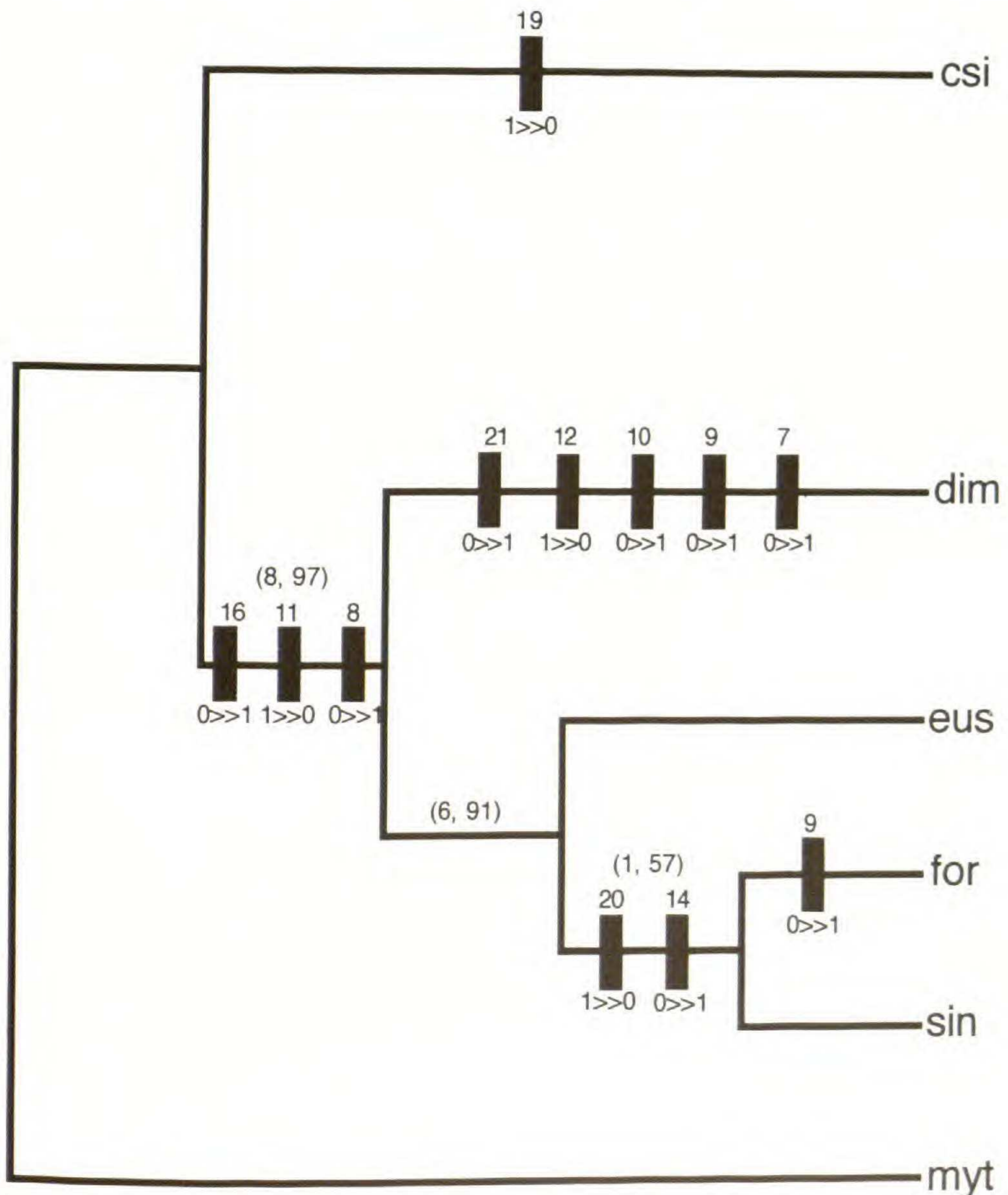


Figure 3. The single most parsimonious tree of 264 steps based on morphology and ITS data. CI = 0.894, RI = 0.52. Taxon abbreviations as in Table 1. Numbers in parentheses are decay index values and bootstrap percentages, respectively. Black rectangles represent the unambiguous changes of morphological characters. Characters and their states are shown in Table 2.

DISCUSSION

Sequence characteristics. The ITS DNA sequences from available angiosperms have shown that ITS-1 and ITS-2 range from 187 to 298 and 187 to 252 bases, respectively, and that ITS-1 is generally larger than ITS-2 (Baldwin et al. 1995). In the

Corylopsis complex, sequence lengths of the ITS region are generally within this range; however, in *Eustigma*, *Fortunearia*, and *Sinowilsonia*, the sizes of ITS-1 and ITS-2 are nearly equal because these three genera share a 36-base deletion in ITS-1 that shortens this spacer. GC contents for ITS-1 and ITS-2 in subfamily Maloideae (Rosaceae) were found to be toward the high end of the range for angiosperms (Campbell et al. 1995). This is true also for the genera of the *Corylopsis* complex (Table 3). The pairwise sequence divergences of the genera are slightly higher in ITS-1 than in ITS-2, but the statistical analysis shows that the difference is not significant ($P = 0.8$). This result agrees with previous studies (Baldwin et al. 1995; Campbell et al. 1995). As expected from the results found in most angiosperms (Baldwin et al. 1995), ITS-1 is slightly more informative than ITS-2 in the *Corylopsis* complex. The trees generated from the two spacers are mostly congruent (Figure 1a, b); therefore, the pooled data produced a phylogeny with at least the same level of resolution as ITS-1 (Figure 1c).

Phylogenetic relationships. The pooled ITS-based phylogeny (Figure 1c) clearly indicates that the *Corylopsis* complex is composed of two well-differentiated clades. The first clade is of *Corylopsis* and the second clade consists of *Distylium* and *Eustigma-Fortunearia-Sinowilsonia*. *Corylopsis* is seemingly similar to *Fortunearia* in leaf morphology; therefore, it has been treated, together with *Fortunearia*, as belonging to the tribe Corylopsi-deae (Chang 1979; Harms 1930; Schulze-Menz 1964). However, *Corylopsis* differs greatly from *Fortunearia* in its semi-evergreen, shrubby habit, bisexuality, and broad, showy petals. Therefore, the phylogeny based on ITS data is consistent with a group of floral characters. The results support the hypothesis that *Corylopsis* is not closely related to any of the other genera in the complex (Endress 1989a, b). Endress (1989a, b) recognized the genus *Corylopsis* as a monogeneric tribe. We believe, however, that this treatment cannot be fully evaluated until a study including a broader range of genera is conducted (Li et al., unpubl. data).

The clade of *Distylium* species is sister to the clade of *Eustigma-Fortunearia-Sinowilsonia* in the trees generated using ITS data (Figure 1a, b, c), but the morphology-based tree (Figure 2) suggests that *Eustigma* is sister to *Distylium-Fortunearia-Sinowilsonia*. However, in the morphology tree, clade support is weak

for the relationship. Therefore, the combined analysis using ITS and morphology (Figure 3) seems to be appropriate (de Queiroz 1993). The resulting tree shows the same tree topology as the ITS phylogeny. Thus, as proposed by Schulze-Menz (1964) and supported by Endress (1989a, b), *Sinowilsonia* is not closely related to *Distylium*.

The clade of *Eustigma-Fortunearia-Sinowilsonia* is strongly supported with a bootstrap value of 91% and a decay index of six steps in the phylogeny based on the combined data set of morphology and ITS. Interestingly, these three genera also share a unique long deletion of 36 bases (Table 4). Therefore, the three genera are undoubtedly closely related, not distantly allied as described by Endress (1989b). However, neither the clade of *Eustigma-Fortunearia* nor the clade of *Sinowilsonia-Fortunearia* is very strongly supported by decay indices and bootstrap values (Figure 1a, b, c). Both *Fortunearia* and *Sinowilsonia* are monotypic, and *Eustigma* has debatably only several species; thus, some taxa bridging them are possibly missing. This might contribute to the loose relationships among them. Morphologically, *Eustigma* is characterized by its greatly expanded purple stigma; as a result, it has been recognized as a monotypic tribe (Chang 1979; Harms 1930). Recently, Endress (1989a, b) put this genus into a tribe containing *Fortunearia* and *Sinowilsonia* based on the fact that *Eustigma* shares reduced petals with *Fortunearia* and *Sinowilsonia* and has lenticellate capsules in common with *Fortunearia*. ITS data in this paper offer strong support for this hypothesis.

Bogle and Philbrick (1980) and Endress (1989c) pointed out that floral structures of the *Corylopsis* complex appear to have become reduced for wind pollination in the course of evolution. The phylogenetic tree based on the combined ITS and morphology data sets (Figure 3) shows a similar picture of character evolution when morphological character states are mapped on the tree. That is, flowers evolved from bisexual (*Corylopsis*) to andromonoecious (*Distylium*, *Fortunearia*) to monoecious (*Sinowilsonia*); petals gradually became reduced from broad and showy in *Corylopsis* to inconspicuous in *Eustigma* and filament-like in *Fortunearia* and *Sinowilsonia*, to completely absent in *Distylium*; pollen grains, however, showed a tendency to increase the number of apertures from three in *Corylopsis* to four in *Distylium*; and flower parts evolved from a fixed number in *Cory-*

lopsis (5), to slightly variable in *Fortunearia* and *Sinowilsonia* (mostly 5 or rarely 6), or to much more variable in *Distylium* (4–8 stamens).

The reconstructed phylogeny of the *Corylopsis* complex based on both morphology and ITS DNA sequences (Figure 3) suggested that: 1) *Corylopsis* should be separated from the other genera; 2) It is justifiable that *Eustigma* be put in the clade of *Fortunearia* and *Sinowilsonia*; and 3) *Sinowilsonia* is more closely related to *Fortunearia* than to *Distylium*.

This study contributes to a better understanding of the intergeneric relationships in the *Corylopsis* complex. However, the tribal relationships in the Hamamelidoideae remain unclear. This is the focus of our ongoing research.

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